DOCKET NO: 239707US0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
MANABU SATO, ET AL.	: EXAMINER: FERNANDEZ, S.
SERIAL NO: 10/609,401	:
FILED: JULY 1, 2003	: GROUP ART UNIT: 1651
FOR: A PROCESS FOR PREPARING AN IMMOBILIZED ENZYME	:

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Now comes Mr. Manabu Sato who deposes and declares that:

1. I am a graduate of Muroran Institute of Technology and received my masters degree in the year 1991.

2. I have been employed by the Kao Corporation for the past 14 years, as a researcher in the field of chemical engineering.

3. I am a named inventor of the above-identified application.

4. The following experiments were conducted by me or under my direct supervision and control.

Comparison Of Examples Between Claimed Invention And U.S. 6,716,610

100 g(dry-weight) Duolite A-568 (Rohm & Haas Co.) as a carrier to immobilize a lipolytic enzyme was stirred for 1 hour in 1 L of 1/10 N NaOH. After filtration, it was

washed with 1 L of deionized water and the pH was equilibrated with 1L of 500 mM phosphate buffer (pH 7). Thereafter, the pH was equilibrated 2 times by a unit of 2 hours with 1 L of 50 mM phosphate buffer (pH 7). Thereafter, the carrier was recovered by filtration and then 500 ml of ethanol was used to replace the solvent by ethanol for 30 minutes. After filtration, 500 ml of ethanol solution containing 100 g of ricinoleic acid come onto contact with the carrier for 30 minutes. Thereafter, the carrier was recovered by filtration and washed 4 times by a unit of 0.5 hours with 500 ml of 50 mM phosphate buffer (pH 7) to remove the ethanol. After the carrier was recovered by filtration, the carrier was brought into contact for 4 hours with 1000 ml of 10% commercial lipase solution (Lipase OF, Meito Sangyo Co., Ltd.) as the enzyme was adsorbed onto the carrier. After adsorption, the carrier having the enzyme was filtrated and washed for 0.5 hours with 500 ml of 50mM phosphate buffer (pH 7). Thereafter, the immobilized enzyme was recovered by filtration. The amount of residual moisture of the immobilized enzyme at this time was 168% to the carrier weight. The amount of canola oil in the following table was added to the immobilized enzyme and the immobilized enzyme in canola oil was stirred for 24 hours at 40°C. Thereafter, the immobilized enzyme was separated by filtration from the oil. The amount of the residual moisture in the immobilized enzyme to the carrier weight and to the immobilized enzyme was estimated. The result was shown in the following table.

	Example	Oil amount for contacting with the immobilized enzyme (wt.% based on weight of the carrier)	The amount of oil in the immobilized enzyme after filtration (wt.% based on weight of the carrier)	Moisture content	
				* (wt% based on the weight of the carrier)	**(wt.% based on the weight of the immobilized enzyme
US 6,716,610	Example 1	400	150	66	21
Claimed Invention	Newly measured data	500	150	52	17
		800	150	42	14
	Example 1	1000	150	29	11

*: the method for counting the moisture content in the immobilized enzyme on this specification

**: the method for counting the moisture content in the immobilized enzyme on reference, USP 6.716,610

I declare under penalty of perjury under the laws of the United States of America that the foregoing is believed to be true and correct.

Manabu Sato

Manabu Sato

May 24, 2005

Date